# DEVELOPMENT AND FORMULATION OF EFFECTIVE BIOFERTILISERS FOR MANAGEMENT OF BLACK PEPPER AND CARDAMOM

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Research Guide


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**1. INTRODUCTION**

Biofertilisers are preparations containing cells of microorganisms which may be nitrogen fixers, phosphorus and potassium solubilizers, sulphur oxidizers or organic matter decomposers. Biofertilisers provide biological nitrogen to plants, provide insoluble phosphorus available to the plants, mobilize potassium and enhance nutrient uptake and plant growth. They also provide protection from soil borne pathogens and help in the survival of beneficial microorganisms. Besides, they improve soil property and maintain soil fertility which indirectly increases the crop yield. N-fixing, P solubilising and K- mobilizing bacteria are important in plant nutrition, increasing NPK uptake by the plants and playing a significant role as PGPR in the bio fertilisation of crops. Biofertilisers form an integral part of Integrated Plant Nutrient Supply system (IPNS) and organic farming which constitutes the present as well as future mandate of Indian agriculture. Indiscriminate use of agrochemicals led to several agricultural and socio-economic problems such as poor cropping system, increased disease incidence and environmental pollution besides exorbitant cost of the products. The excessive application of chemical nitrogen fertilizer accelerated soil acidification and threatened

air and groundwater contamination at the same time. Thereby to overcome these ecological problems, we are now directing ourselves towards the potential use of ecofreindly approaches such as the use of biofertilisers to sustain high production, allow more efficient nutrient utilization and thereby provide solutions for present and future agricultural practices.

For any microbial inoculants in agriculture, an efficient formulation is essential to translate laboratory activity into adequate field performance. A major role of inoculants formulation is to provide more suitable microenvironment for the prolonged survival of organisms in the soil. Formulations based on polymers, especially alginate, have received considerable interest in recent times. Little progress had been made in this area despite the fact that formulations will improve the field efficiency of fungal BCAs and expand their market opportunities. However although bacterial cells in carrier materials introduced into the soil may show enhanced survival and effectiveness, additional soil treatments intended to cause shifts in the soil microbiological balance will often be necessary to improve the effectiveness further. Appropriate formulation and developing methods are critical for efficiency as well as shelf life of bio-fertilizers. The present work aims to develop a suitable biofertilizer formulation for black pepper and cardamom by exploiting the natural indigenous microbial wealth.

**2. IMPORTANCE OF THE STUDY**

Spices are highly export oriented crops and it is mandatory that the produce as well as the products from them should be in pesticide free condition as a bio-safety measure. The production of spices can be increased considerably through integrated nutrient management. But as the extent of cultivation is increased, the amount of inorganic fertilisers and pesticides reaching the soil strata are found to be beyond the residue limit. Black pepper (*Piper nigrum* L.), called as the “King of Spices” belongs to Piperaceae is one of the oldest and best-known spice in the world. In India there are only few states like Kerala, Karnataka, Tamil Nadu, and Andaman and Nicobar Islands that produce black pepper. Similarly small cardamom (*Elettaria cardamomum* M.) often referred as the “Queen of Spices” belongs to the family Zingiberaceae is the third most

expensive spice in the world, after Saffron and vanilla. It grows as a native in the southern Indian forests of the Western Ghats. It is also grown in Sri Lanka, Guatemala, Indo China and Tanzania.

The management practices in these crops include application of chemical fertilizers and plant protection chemicals which become inevitable to the crop due to the depletion of soil organic matter and increased incidence of pests and diseases. But the extensive use of inorganic chemicals deteriorated the soil health which in turn affected the productivity of the crops. So in order to revitalize the health of the soil and to increase the productivity, it is inevitable to enrich the organic content of the soil through the use of microorganisms. Already a number of experiments have been conducted to study the effect of biofertilizers including N-fixers and phosphate solubilizers in black pepper and cardamom. But many of this work have been done only with individual or exotic strains of the organisms. The lacunae observed in these crops are

1) Lack of exploitation of indigenous micro flora having potential of Nitrogen fixation, Phosphate solubilization or Potash mobilization along with bio protective properties.
2) Lack of suitable delivery system to discharge the potential biofertilisers in a consortia mode.

Based on these informations, the objectives of the present study are

**3. OBJECTIVES**

- Isolation, screening and characterization of indigenous strains of $N_2$ fixers, Phosphate solubilizers and K mobilizers from black pepper and cardamom rhizosphere / ecosystems.
- Evaluation of the efficacy of potential strains for growth promotion and for biological protection from soil borne pathogens.
- Development and formulation of bio-fertilizer consortia with the potential putative isolates in a suitable, stable, eco-friendly and economical delivery system.

## 4. REVIEW OF LITERATURE

Biofertilisers provide nutrient sources and good soil conditions for the growths of crops, to partially substitute and enhance the function of chemical fertilizer and then subdue the application quantities of fertilizers and to lessen the negative effect aroused from applying chemical fertilizers to soil. The other purposes of using bio-fertilizers to soil are to enhance the growth of root system to increase the water and nutrient absorption abilities of crops, neutralize and degrade harmful materials accumulated in soil and also to promote survival efficiency of seedling after transplanting and get shorter time for the flower to come out (Chiu-Chung Young, 2005). By 2030, world population shall increase by about +33 to +67%, while in the mean time, the potential increase in surface area of arable land will reach at most +20%, and that of irrigated land less than +10% (Vance *et al*. 2003). So there is a need to increase the level of agriculture productivity in a sustainable manner. To overcome these ecological problems and to minimize the use of chemical fertilizers we are now directing ourselves towards the potential use of ecofreindly approaches such as the use of Biofertilizers and Biopesticides to sustain high production, more efficient nutrient utilization and thereby finding solutions for present and future agricultural practices (Kennedy, 1998; Bowen and Rovira, 1999; Barea *et al.* 2004; Lucy *et al.* 2004). Certain co-operative microbial activities can be exploited as a low-input biotechnology, and form a basis for a strategy to help sustainable, environmentally-friendly practices fundamental to the stability and productivity of both agricultural systems and natural ecosystems (Kennedy and Smith, 1995). Microbial inoculum not only increased the nutritional assimilation of plant, but also improved soil properties, such as organic matter content and total nitrogen in soil (Wu *et al*. 2005).

Free nitrogen-fixing bacteria were probably the first rhizobacteria used to promote plant growth. Azospirillum strains have been isolated and used ever since the 1970s when it was first used. The study by Bashan *et al.* (2004) being the most important one reporting the latest advances in physiology, molecular characteristics and agricultural applications of this genus. Other bacterial genera capable of nitrogen fixation and

growth promotion effect are *Azoarcus* sp., *Burkholderia* sp., *Gluconacetobacter diazotrophicus*, *Herbaspirillum* sp., *Azotobacter* sp and *Paenibacillus polymyxa*. These strains have been isolated from a number of plant species such as rice, sugarcane, corn, sorghum, other cereals, pineapple and coffee bean (Sompong *et al.* 2004). Plant beneficial effects of *Azospirillum* have mainly been attributed to the production of phytohormones, nitrate reduction and nitrogen fixation, which have been the subject of extensive research. Due to their frequent occurrence in the rhizosphere, these are known as associative diazotrophs (Somers *et al.*2004; Bashan and de Bashan, 2005; Baldani *et al.* 2005; Helsel *et al*.2006; Fallik, 1994).

Indian soil contains on average 0.05% phosphorus which constitutes 0.2% dry weight. (Shrivastav *et al*. 2004). Plants are only able to absorb the soluble forms, that is, mono- and dibasic phosphate. Besides inorganic forms of phosphorous in soil, the phosphorous present in organic matter is of considerable importance. The organic forms of phosphorous are estimated to comprise between 30 and 50% of total soil phosphorous. This reservoir can be mineralized by microorganisms, making it available to the plant as soluble phosphates. Many bacteria from different genera are capable of solubilizing phosphate. Most of these bacteria are able to solubilize the Ca–P complex, and there are others that operate on the Fe–P, Mn–P and Al–P complexes. Knowledge of their mechanisms and ecology in the rhizosphere will improve their use in sustainable agriculture (Gyaneshwar *et al*. 2002). Beneficial effects of the inoculation with P-solubilizing bacteria to many crops plants have been described by numerous authors. Also there are several reports on inorganic phosphate solubilization by *Pseudomonas* sp. (Jagadesh, 2003; Deepa *et al*. 2003; Arti *et al*. 2003, Hui *et al*. 2011; Prasanna *et al*. 2011). Two phosphate solubilizing biofertilizers in combination with different levels of P increased plant growth and nutrient uptake of sunflower (Ravi and Algawadi, 2003). Rock phosphate with phosphate solubiliser (*Bacillus polymyxa*) significantly increased the green chilli yield (Patil and Madalgeri, 2003).

Potassium mobilizer mediated increase in potassium uptake has been recorded by Chandra and Singh (1999). Similarly Nayak *et al*. (2001) studied the effect of potash

mobilizer on brinjal and recorded increased potash uptake and increased plant biomass in potash mobilizer treated plants as compared to control. Sheng (2005) examined the plant growth promoting effects and nutrient uptake of potassium releasing strain *Bacillus edaphicus* NBT on cotton and rape. Similarly the application of biofertilisers containing N fixer (*Azotobacter chroococcum*), P solubilizer (*Bacillus megaterium*) and K solubilizer (*Bacillus mucilaginous*) and AM fungi (*Glomus mosseae*) significantly increased the growth of *Zea mays.* Studies on the effect of potash solubilizing/mobilizing bacteria *Frateuria aurantia* on brinjal showed significant increase in yield, plant height and potassium uptake (Ramarethinam and Krishan Chandra, 2006). Similarly, there are also reports where soil inoculation with PSB or KSB significantly increased N, P and K uptake in pepper and cucumber plants (Han *et al*. 2006).

The nutritional requirements of black Pepper are to be considered in the light of agro ecological conditions, soil nutrient status and incidence of pests and diseases that affect yield to a great extent. Nitrogen, Phosphorus, potassium, magnesium and micronutrients are the most important nutrients for growth, development and yield of black pepper and their influence depend on their ratios in the soil as well as in the plant. Adequate manuring is necessary for proper vine growth and yield of black pepper. The quality of NPK fertilizers required to get high yield vary largely among locations (Sadanandan, 1994). Nybe *et al*. (1989) reported that phosphorus and potassium had greater importance in enhancing the yield. Major black pepper growing states of south India in 300 locations showed that the soils are intensively leached (2000-4000mm rain in 120-140 rainy days $year^{-1}$) and deficient in P, K, Ca, Mg and Zn (Hamza *et al*. 2007). Among the several factors responsible for low yield in cardamom, inadequate application of fertilizers or manures and dry spells are the major ones. Korikanthimath (2000) emphasized that application of organic and inorganic nutrients is essential as a part of scientific management that helps in maintenance of soil health and production of the crop. A significantly positive correlation between organic carbon and total as well as available N was observed by Srinivasan (1984). Soil test results of cardamom growing areas have indicated that majority of soils are low to medium in available P (Srinivasan

and Mary,1981; Thomas and Kuruvila, 2007). Although the available K appears to be high as judged by the traditional soil test methods, Ranganathan and Natesan (1985) opined that it is intrinsically low as the soil tests estimate only the exchangeable form of potassium which is highly mobile and it could either be taken up by the plants or lost by leaching.

There are also indications that black pepper plants can benefit from the use of biofertilizers. Beneficial effect of *Azospirillum* inoculation has been reported in black pepper (Govindan and Chandy, 1985; Bopaiah and Khader, 1989; Kandiannan *et al*. 2000). Application of *Azospirillum* and chemical fertilizers NPK in addition to farm yard manure resulted in high dry berry yield (Kanthaswamy *et al*. 1996). Higher values of all the soil nutrients of black pepper were exhibited with the application of organic and biofertilizer with inorganic combinations (Nybe and Filitte, 2003). It was reported that significant uptake of N, P and enhanced vigour of black pepper when inoculated with *Pseudomonas fluorescens* strains, due to their high root proliferation and nutrient mobilization in the rhizosphere (Diby *et al*. 2005). The black pepper yield has improved by application of *Azospirillum* 50 g and Phosphobacteria 50 g with recommended 100% NPK with 10 kg farm yard manure, followed by 50 g *Azospirillum* with 75% recommended NPK (AICRPS, 2007). Phosphobacterial isolates from the rhizosphere soil and root cuttings of bush pepper showed growth promoting activity (Kavitha *et al*. 2007). **However, reports are very scanty on the use of indigenous native isolates to black pepper.**

*Phytophthora capsici* is a soil borne pathogen and the causal agent of foot rot of black pepper (Anith *et al*. 2002). The disease has been found in major black pepper growing areas. Black berry disease (Pollu disease) is one of the most common fungal diseases on black pepper and is caused by *Colletotrichum gloeosporioides* . It limits berry production and causes significant yield losses. Similarly basal rot caused by *Sclerotium rolfisii* is also a major problem in black pepper nursery (Sarma *et al*. 2000). Anandaraj (2000) reported that in the rhizosphere of black pepper, several antagonistic microorganisms occur. Studies on the biological control of foot rot are mainly focused on identifying

microorganisms from the rhizosphere of black pepper that can effectively suppress foot rot. Diseases such as rhizome rot and leaf spot caused by pathogenic fungi such as *Pythium vexans* de Barry, *Rhizoctonia solani* Kuhn limits large scale production of cardamom (Thomas and Suseela Bhai, 1990). *Trichoderma harzianum* reported to reduce rhizome rot incidence in cardamom plantations (Thomas *et al*. 1991). A formulation of *T.harzianum* in a carrier medium consisting of FYM and coffee husk has been developed (Thomas *et al*. 1997).

There are already reports of combined inoculations with different biofertilizers to replace or reduce the quantity of inorganic NPK under INM. Combined inoculation of *Azospirillum brasilense* and the phosphate-solubilizing bacteria *Pseudomonas striata* or *Bacillus polymyxa* on field-grown sorghum was reported by Alagawadi and Gaur (1992). inoculation of mixed microbial consortium of *Azotobacter, Azospirillum,* Phosphate solubilizing bacteria, Plant growth promoting rhizobacteria, Arbuscular mycorrhizal fungi maximum per cent of nitrogen uptake (2.205%) and gave highest dry chilli yields of 3409.7 kg/ha with a saving of 25% of chemical fertilizers (Trimurtulu *et al*. 2011).The influence of combined inoculation of 12.5 g *Azospirillum*, 12.5 g *Psuedomonas fluorescens* and 10 g phosphobacteria/kg of seed was studied in rice cv. IR 50. Increases in plant vigour and dry biomass were found in inoculated treatments compared with controls( Kamble *et al*. 2011).

Researches on the formulation of biofertilizers are limited. Somasegaran (1985) demonstrated the potential of using diluted cultures of *Rhizobium sp.* with autoclaved and gamma irradiated peat for inoculants preparation. Bahme *et al.* (1988) developed two new delivery systems, granule application and low-pressure drip irrigation delivery for applying rhizobacteria to underground organs of potato. Fly ash and its different combinations with soil as a carrier showed maximum viability for diazotrophs and phosphobacteria (Gaind, 2004). New forms of dehydrated biofertilisers (big capsules) with *Azospirillum brasilense* have been developed for application in cereal fields (Ivanova *et al*. 2002). Ahmet *et al*. (2003) reported that spraying a growth promoting bacteria, *Bacillus*-OSV142, significantly increased the yield, growth and nutrient element

composition of leaves of apricot. Preininger *et al*. (2003) developed biolistic method for the direct introduction of nitrogen fixing bacteria (*Azotobacter vinelandii*) into the strawberry tissues. Viveganandgan and Jauhri (2000) reported the superiority of alginate based formulations over charcoal based ones in maintaining the population of two phosphate solubilizing bacteria during storage. A method using alginate micro beads as a substrate and *Azospirillum brasilense* as the model PGPB was developed by Bashan *et al* (2002). Rekha *et al.* (2007) demonstrated that inoculation of encapsulated bacterial isolates promoted plant growth similar to their respective free cells and could be a novel and feasible technique for application in agriculture. Sodium alginate based formulations of two plant growth promoting bacteria, *Bacillus subtilis* and *Pseudomonas corrugata* retained plant growth promoting ability, root colonization, antifungal and enzyme activities after three years (Pankaj and Anita, 2008). Power *et al*. (2011) showed that alginate beads are an effective storage and delivery system for PCB degrading *Pseudomonas fluorescens and* after 250 days of storage, 100% recovery of viable cells was possible. But no such work on formulations has been done in black pepper or cardamom.

## 5. MATERIALS AND METHODS

### 5.1. Isolation and screening of indigenous strains of N fixers, P and K solubilizers from Black pepper and Cardamom rhizosphere / ecosystems.

#### 5.1.1. Collection of soil samples from healthy Black pepper and Cardamom growing tracts of Kerala and Karnataka.

Sample collection was carried out from unexplored black pepper and cardamom growing tracts of Kerala and Karnataka. Representative samples were collected from each location mainly from biologically active soil (like forest soil) expected to contain a diverse group of microorganisms. The samples were collected in polythene bags and kept in ice-box and brought to the laboratory and stored at 4 °C until processed.

#### 5.1.2. Isolation of indigenous N-fixers, P and K solubilizers.

Specific group of organisms such as $N_2$-fixers, P solubilizers and K mobilizers were isolated using selective media. Nitrogen fixers and Phosphate solubilizers were isolated

from soil and root samples based on standard methodology (Tarrand *et al*.1978; Enrique 1982; Pikovskaya, 1948; Babana and Antoun, 2005 and Nautiyal, 1999). For the isolation of K-mobilizers, the sucrose minimal salt medium designed by Sheng (2005) was modified and used. The modification was made in such a way that the mineral Illite was replaced by different quantities of wood ash (0.5-2.5%) which was found as effective as illite in isolating K-mobilizers from the soil. Isolated colonies were characterized, sub cultured and maintained for further studies.

**5. 1.3. *In vitro* evaluation of isolates for N fixation, P and K Solubilization**

*In vitro* studies on Nitrogen-fixation, Phosphate and Potassium Solubilization, Indole acetic production, antibiotic sensitivity etc. were done based on standard methodology. Putative nitrogen fixing bacteria were screened in the ACC deaminase defined medium. (Day and Dobereiner, 1976). Nitrogen fixing ability was determined using Nfb medium. The isolates were grown in semisolid medium for 72 h, homogenized, digested with $H_2SO_4$ and the aliquots were estimated for total nitrogen (Jackson, 1973). The Phosphobacteria isolates were screened for their phosphate solubilizing efficiency (Nguyen *et al.* 1992). The concentration of soluble P in the supernatant was determined using chloromolybdic acid blue colour method at 660ηm (Olsen 1982). The available potassium content was measured using atomic absorption spectrophotometer AAS 240FS in emission mode. The Indole acetic acid production (IAA) was determined in L-tryptophan agar using Salkowski reagent (Sarwar and Kremer, 1995).

**5.2. Evaluation of the efficacy of potential strains for growth promotion and for biological protection from soil borne pathogens.**

**5.2.1. Screening of the isolates for its ability to contribute towards plant growth promotion.**

The experiments were conducted at Indian Institute of Spices Research, Experimental Farm at Peruvannamuzhi, Kerala and at Indian Institute of Spices Research, Cardamom Research Centre, Appangala, Karnataka. Rooted cuttings of black pepper in 3-4 leaf stage (IISR Sreekara) and cardamom seedlings of 3-4 leaf stage (IISR Kodagu Suvasini) were used in the experiment. Surface disinfected rooted cuttings/seedlings were root

treated with bacterial cell culture suspension @ $10^8$ cfu $mL^{-1}$ for 15 min and transplanted into polythene bags filled with sterilized potting mixture consisting of soil: farm yard manure: sand (1:1:1). The plants were repeat inoculated with respective bacterial suspension thrice at monthly interval as soil drench. The growth parameters were recorded regularly and destructive sampling was done for estimating the biomass and nutrient analysis. Finally major, secondary and micronutrients were estimated in roots, shoots and soil by standard protocols (Bremner, 1996; Jackson, 1973). Rhizosphere colonization by the isolates was also monitored. The potential isolates were shortlisted for further studies.

**5.2.2. *In vitro* screening of the potential isolates for its biocontrol potential.**

The dual culture plate method was employed to examine the inhibition of rhizobacterial isolates against the major pathogens, *Phytophthora capsici*, *Pythium vexans*, *Colletotrichum gloeosporioides*, *Rhizoctonia solani*, *Sclerotium* sp and *P. meadii.* A 5 mm mycelial plug of the target pathogen was inoculated at the centre of Potato Dextrose Agar (PDA) plate and the bacterial isolates were streaked on either side. The plates were incubated at 28 °C and the diameter of the colony was measured after 72 h. Besides colony diameter, those isolates that produced a clear inhibition zones were also considered as effective.

**5.2.3. Testing the compatibility of the putative organisms with recommended biocontrol agents (*in vitro*).**

The potential isolates were studied for their compatibility with recommended bacterial biocontrol agents such as IISR 6 (*Pseudomonas aeruginosa*), IISR 853 (*Pseudomonas aeruginosa*), 859 (*Alcaligens faecalis*), PB21c (*Serratia marescens*) and $P_1AR_6$ (*Rhizobium rhizogenes*) and *Trichoderma harzianum*. Here bacterial suspensions were made by suspending cells in 10 mM $MgSO_4$. The density was measured spectrophotometrically at 660 nm and adjusted to $10^8$ colony forming units/ml. The putative strains were spot inoculated on nutrient agar plates by pipetting two droplets of 5 µl of the bacterial suspension. The spot inoculated plates were incubated at $30^0$C for 48 h. Subsequently a suspension of the target biocontrol strain ($10^8$ cfu/ml) was atomized over the spot

inoculated plates. After an additional incubation period of 24 h at $28^0$C, zones of growth inhibition of the targeted strain around the spot inoculated strains were measured (Zerela *et al*. 2007; Marjan *et al*. 1999).

**5.2.4. Evaluation of the short listed candidates for growth promotion along with inorganic fertilizers in different consortium mode under nursery Condition.**

The potential isolates were further tested in different combinations under varying levels of NPK fertilizers (0, 50, 75 and 100%) along with the recommended dose (RDF). The common NPK recommendation for black pepper under field condition was 140:55:270 and for cardamom under field condition was 75:75:150. The 50, 75 and 100% levels were calculated based on recommended dose. Nitrogen was applied as urea (46% N), phosphorus as rock phosphate (18% P) and potassium as muriate of potash (60% K). There were 8 treatments each for black pepper and cardamom includes different combinations of short-listed isolates including control with recommended fertilizer dose and absolute control without any fertilizer or organisms.

**5.2.5. Characterization and Identification of the putative organisms using biochemical / molecular techniques.**

The short-listed isolates were identified using **16srDNA sequence** analysis as well as by **BIOLOG ML5** micro station system.

Genomic DNA from the bacteria was isolated according to the protocol of Kumar *et al*. (2004). Amplification of 16s rDNA gene of bacteria was performed with universal primer set pA (Fp) (5'-AgAgTTTgATCCTggCTCAg-3') and pH (Rp) (5'- AAggAggTgATCCAgCCgCA -3') (Woese, 1987; Stackebrandt and Goebel, 1994). The final PCR product was resolved in 0.8% agarose gel, excised and purified with Sigma elution kit. DNA sequencing was performed and sequences were subjected to a BLAST analysis and nucleotide sequence similarities were determined with the NCBI (National Centre for Biotechnology Information databases) and their identity was established by closest match.

The Biolog GEN III micro plate provides a phenotypic fingerprint of the microorganism. The isolates to be identified was grown on agar medium and then suspended in inoculating fluid at recommended cell density. Then the cell suspension was inoculated

into the microplate @ 100 µl per well and incubated. During incubation there is an increased respiration cause's reduction of tetrazolium dye, forming purple colour. After incubation, the phenotypic fingerprints of the purple wells were compared with biologs extensive species library.

**5.3. Development and formulation of a biofertilizer consortium with the potential isolates in a suitable, stable, eco-friendly and economical delivery system.**

**5.3.1. Testing of the different solid and liquid media for long term storage of the consortia.**

The putative bacterial isolates were raised in culture suspension and prepared three formulations: (1) talc (2) broth and (3) sodium alginate beads. The formulations were stored at room temperature and at $4^0$ C in the refrigerator. The viability of bacteria in these formulations were enumerated by dissolving alginate beads in sodium citrate (1%) and talc formulations and broth formulations by adding in phosphate buffered saline, at an interval of 7 days up to a period of 90 days of storage. For encapsulation, inoculums were prepared in a 10ml vial containing 9.8 ml of medium and 0.2 ml of pre culture. The vial was incubated overnight at $30^0$ C on a rotating shaker at 120rpm. This culture was centrifuged for 10min at 8000×g at $4^0$ C. The cells were then washed thoroughly with 10 ml of 0.8% of NaCl solution and re-suspended in 1 ml of peptone solution. Sodium alginate 2% was sterilized separately and dissolved in water for 30 min). The cells were added and mixed homogenously. The isolates were mixed indivilally and as consortium and cell free beads served as controls. The mixture was introduced in a syringe and extruded drop by drop into 1.5% $CaCl_2$. After 30 min, the beads are washed in sterile water and were transferred into fresh nutrient medium for an additional incubation for 12 h at 100 rpm. The wet beads were spread on a filter paper and dried at $30^0$ C for 48 h. The dried beads were collected and stored at five different conditions viz. (1) room temperature, (2) dessicator, (3) $4^0$ C, (4) water and (5) $CaCl_2$ solution.

**5.3.2. Survival of the encapsulated biofertilizer in different organic bases**

Based on survival rate of isolates, the alginate beads were further evaluated in different substrates such as Soil, Vermicompost, Farm yard manure and Neem cake. The

substrates were sterilized separately and the encapsulated beads were mixed to it. The release of bacteria, bead characteristics and biodegradability were examined at regular intervals.

**5.3.3. *In vivo* effect of encapsulated biofertilizer strains with organic manures**

The *in vitro* experiments with vermicompost mixed with encapsulated biofertilizer strains and strains alone give similar results. So another *in vivo* study was based on vermicompost alone as a substrate in addition to soil with different number of encapsulated alginate beads to find out the exact quantity of beads required for enhancing the growth of the plant. There were 9 treatments with 20 plants treatment$^{-1}$. The treatments include 1, 5, 10, 20 and 25 beads respectively in vermicompost along with recommended dose of NPK, vermicompost alone and absolute control with potting mixture.

## 6. RESULTS AND DISCUSSION

### 6.1. Isolation and screening of indigenous strains of N fixers, P and K solubilizers from Black pepper and Cardamom rhizosphere / ecosystems.

**6.1. 1. Collection of soil samples**

A total 80 soil samples were collected from major black pepper and cardamom growing areas. The collection covered wild unexplored areas of Wayand, Thrissur (Chimney forests), Palakkad (Nelliyampathy and Parambikulam forests, High ranges of Idukki and Kannur in Kerala and Coorg district in Karnataka. A few samples were also taken from plantations with not much cultivation practices for the last few years.

**6.1.2. Isolation of indigenous N fixers, P and K solubilizers from healthy Black pepper and Cardamom growing tracts of Kerala and Karnataka**

Thirty six bacterial isolates were shortlisted from a total of 103 isolates including 23 isolates from Black Pepper (11 N fixers, 9 Phosphobacteria and 3 K solubilizers) and 13 isolates from Cardamom (8 N fixers, 2 Phosphobacteria and 3 K solubilizers) were isolated.

**6.1.3 *In vitro* evaluation of isolates for N fixation, P and K solubilization**

The black pepper isolates showed wide variations in their *invitro* nitrogen fixing abilities ranging from 3.2 to 8 mg N $g^{-1}$of malic acid. The N-fixation capacity of the isolates IISRBPaz4 and IISRBPaz9 tested *invitro* showed 8.68 and 8.52 mg N $g^{-1}$ malate. The Phosphobacterial isolates showed solubilization activity ranging from 57.1(IISRBPb7) and 80.6 (IISRBPb2) mg P/l. The potassium solubilizers showed solubilizing ability between 165 and 256 mg K/l. The N fixation capacity of the Cardamom isolates IISR Caz3 and IISRCaz9 tested *in vitro* showed 7.00 and 6.72 mg N $g^{-1}$ malate. The phosphobacterial isolates IISR CPb1 and IISRCPb2 showed solubilization activity of 57.0 and 73.7 mg P/l. The potassium solubilizers showed mobilizing ability between 194 and 321 mg K/l.

**6.2. Evaluation of the efficacy of potential strains for growth promotion and for biological protection from soil borne pathogens.**

**6.2.1. Screening of the isolates for its ability to contribute towards plant growth promotion**

The isolate IISRBPaz9 inoculation produced total biomass of 9.75 g $plant^{-1}$ followed by IISRBPaz4 (9.74 g $plant^{-1}$) which is 47% increase over the control. Maximum nitrogen uptake was shown by the isolate IISRBPaz4, followed by IISRBPaz9 (33 and 43% respectively compared to control). The isolates BPaz4 and BPaz9 also showed better colonization in different portions of root. The Phosphobacterial isolate IISRBPb4 showed maximum total biomass production (75% over control), followed by BPb8 (48% over control). The increasing N, P, K and Ca uptake was more pronounced with IISR BPb4, followed by BPb8. The isolate IISRBPb2 showed highest colonization ($86.5x10^4$ cfu/g), when compared to other isolates. Potassium mobilizing isolate IISRBK2 showed higher dry matter content, high Potassium content in the plant and increased K uptake. The isolate also showed maximum colonization in the rhizosphere soil.

There is three-fold increase in the dry weight of the cardamom seedlings in treatments with IISRCaz3 (7.0g) and IISRCaz9 (6.52g), when compared to control (2.35g). NPK and Ca, Mg uptake was significantly higher in Caz3 followed by Caz9. The isolates Caz3 and Caz9 also showed better colonization in different portions of root. The K mobilizing

isolate IISRCK1 showed increased biomass and K uptake in cardamom compared to other isolates.

### 6.2.2. *In vitro* screening of the potential isolates for its biocontrol potential

*In vitro* evaluation against *Phytophthora capsici* (05-09) revealed that none of the nitrogen fixers and potassium solubilizers has disease suppressive effect. The *Pseudomonas fluorescens* (IISRBPb2) isolate is found suppressive *P. capsici* (60% inhibition) indicates twin properties of promoting plant growth and disease suppression. Similarly, *Azosprillum lipoferum* (IISRBPaz9) was able to control *Colletotrichum gloeosporioides* (60% inhibition). The isolate *Azospirillum brasilense* IISRCaz9 showed 69% suppression against *Rhizoctonia solani*.

### 6.2.3. Testing the compatibility of the putative organisms with recommended biocontrol agents (*in vitro*)

The cardamom isolates are compatible among themselves and also with recommended biocontrol agent's viz**.** IISR6, 853, 859, PB21c and P1AR6. The black pepper isolates were compatible with each other except IISR BPaz4 with BPb8 and IISRBPb8 with IISR 853. The black pepper isolates were also compatible with cardamom isolates.

Based on biological characterization, six indigenous isolates of black Pepper including IISRBPaz4, BPaz9 (N fixing), IISRBPb2, BPb4 and BPb8 (P solubilizing) and IISRBK2 (K solubilizer) and four indigenous isolates of cardamom including IISRCaz3, Caz9 (N fixing), IISRCPb2 (P solubilizing) and IISRCK1 (K solubilizer) were short listed.

### 6.2.4 Evaluation of the short listed candidates for growth promotion along with inorganic fertilizers in different consortium mode under nursery condition.

Considering the overall data for black pepper, recommended dose of NPK @ 75 % with IISRBPaz9 (N fixer) + IISRBPb4 (P solubilizer) + IISRBK2 (K solubilizer) combination was found superior in growth promotion, biomass, NPK uptake and rhizosphere colonization followed by same combination at zero level.

Considering the overall data for cardamom, half the recommended dose of NPK fertilizer with IISRCaz9 (N fixer) + IISRCPb2 (P solubilizer) + IISRCK1 (K solubilizer) showed

better growth of cardamom. The combination gives increased plant height, number of tillers, root length, total dry weight and higher uptake of nitrogen, phosphorus and potassium.

**6.2.5 Characterization and Identification of the putative organisms using biochemical / molecular techniques**

The short listed isolates were identified as *Azosprillum lipoferum* (IISRBPaz4 and IISRBPaz9), *Pseudomonas fluorescens* (IISRBPb2), *Bacillus subtilis* (IISRBPb4), *Bacillus cereus* (IISRBPb8), *Paenibacillus glucanolyticus* (IISRBK2), *Agrobacterium tumefaciens* (IISRCaz3), *Azospirillum brasilense* (IISRCaz9), *Acinetobacter baumanni* (IISRCPb2) and *Bacillus* sp.( IISRCK1)

**6. 3. Development and formulation of a biofertiliser consortium with the potential isolates in a suitable, stable, eco-friendly and economical delivery system.**

**6.3.1 Testing of the different solid and liquid media for long term storage of the consortia**

Formulations studies revealed that survival rate of isolates were higher in alginate beads compared to talc and broth. Both individual and mixed combinations of beads showed higher microbial count after 180 days. The black pepper isolates of *Azospirillum lipoferum* (N fixer), *Bacillus subtilis* (P solubilizer) and *Paenibacillus glucanolyticus* (K solubilizer) showed higher survival rate both individually and in consortia mode in encapsulated sodium alginate beads. Similarly for cardamom the isolates, *Azospirillum brasilense, Acinetobacter baumannii* and *bacillus* sp. also showed maximum survival in consortia as in the case of black pepper isolates.

Among the different storage conditions, beads stored at room temperature and in dessicator at room temperature and beads stored in water recorded higher survival compared to beads at $4^0$C and $CaCl_2$ solution. However, water acts as a releasing media for the bacteria from the beads and ultimately results in bead disintegration.

**6.3.2 Survival of the encapsulated biofertilizer in different organic bases**

The alginate beads in different substrates showed that beads with vermicompost and soil recorded higher release rate and survival of the bacteria. The beads completely

disintegrated in neem cake within 14 days. But no active microbial population was found.

**6.3.4. *In vivo* effect of encapsulated biofertilizers with vermicompost**

In black pepper, among the 9 treatments, vermicompost with 25 encapsulated beads (1g of beads) showed maximum plant height (55.2cm), number of leaves (8.8), soil available N (146Ppm) and P (13.3Ppm) when compared to other treatments, followed by vermicompost with 20 beads with higher root length (23.4cm) and soil K (431Ppm). Vermicompost with 20-25 biofertilizer encapsulated beads favors the maximum colonization of isolates. In cardamom also the same trend was observed as in black pepper. The height of the plant (52.7cm), soil available N (159Ppm), P (2.22Ppm), K (345Ppm) and dry weight (3.7g) were higher in the treatment with 25 encapsulated beads and was comparable to 20 beads. **Vermicompost with 20-25 encapsulated alginate beads (1g) were found optimum to enhance the growth and overall performance of black pepper and cardamom in the nursery.**

**7. CONCLUSIONS**

- 36 indigenous biofertilizer bacterial isolates having the potential of N fixation, P and K solubilization consisting of root endophytes and rhizospheric inhabitants were isolated from different black pepper and cardamom growing tracts of Kerala and Karnataka.
- The efficient indigenous isolates include *Azospirillum lipoferum*, *Pseudomonas fluorescens, Bacillus subtilis*, *Bacillus cereus*, *Paenibacillus glucanolyticus*, *Agrobacterium tumefaciens, Azospirillum brasiliense*, *Acinetobacter baumannii* and *Bacillus* sp. Some of the identified isolates of efficient indigenous biofertilisers are reported for the first time in black pepper and cardamom.
- **This is the first report of K solubilizing bacteria in black pepper and cardamom**.
- **The sucrose minimal salt medium was modified by replacing the mineral illite with wood ash**, a low cost, affordable and adoptable organic and inorganic

material and a readily available K source. This is a **novel approach for the isolation of K solubilizing bacteria.**

- **The efficient K solubilizing isolate from black pepper IISRBK2 was identified as *Paenibacillus glucanolyticus*** and IISRCK1 was identified as *Bacillus* sp. There are reports on K solubilization by these species in other crops.
- Two out of 11 indigenous isolates of N fixers (*A.lipoferum* IISRBPaz4 and IISRBPaz9) showed significant increase in shoot and root growth, dry matter accumulation and nutrient content in black pepper over uninoculated control. Higher nutrient uptake in IISRCaz3 and IISRCaz9 can be related to the inoculation effects by *A.tumefaciens* and *A. brasilense* which stimulated root growth and development of the host plants.
- Certain isolates also showed antagonistic properties towards pathogens such as *Phytophthora capsici, Colletotrichum gloeosporioides, Rhizoctonia solani* and *Phytophthora meadii*. The efficient indigenous P solubilizing *Pseudomonas fluorescens*, IISRBPb2 was found to suppress *P. capsici,* the causative agent of foot rot of black pepper. The efficient indigenous N fixing isolate *A. lipoferum*, IISR BPaz9 inhibited *C. gloeosporioides*, the causative agent for anthracnose disease of black pepper. The indigenous cardamom isolate *A. brasilense*, IISRCaz9 showed antagonism against multiple pathogens such as *R. solani, P.meadii and P.capsici.* The study revealed the attractive possibility and versatility in growth and nutrient absorption and biological control by specific indigenous isolates.
- For black pepper, the efficiency of shortlisted isolates with different levels of inorganic fertilizers clearly indicated that N status of the soil with organisms alone is comparable to the status at 75% NPK level which showed the potential of these organisms in fixing atmospheric N which is available for uptake. For cardamom, based on the consortia performance and overall performance, the consortia holding *A.brasilense* + *A. baumannii* + *Bacillus sp.* at 50 and 75% recommended dose of NPK fertilizer showed better growth.

- Of the different formulations tested like talc and broth, alginate bead was found as a suitable carrier media for delivering the biofertilisers into the rhizosphere.
- The black pepper isolates of *Azospirillum lipoferum (*N-fixers*)*, *Bacillus subtilis* (P. solubilizers) and *Paenibacillus glucanolyticus* (K solubilizer) and cardamom isolates *Azospirillum brasilense, Acinetobacter baumannii* and *bacillus* sp. also showed maximum survival in consortia in encapsulated beads.
- Among different organic substrates such as vermicompost (VC), farm yard manure (FYM) and neemcake (NC) tested in comparison with soil; VC supported maximum growth of black pepper and cardamom isolates.
- Suitable and viable storage condition for long term storage of the biofertilizer encapsulated bead was also standardized. Among these, beads stored at room temperature in glass vials, in desiccators and in water recorded higher survival.
- The quantity of encapsulated beads required for promoting black pepper and cardamom growth in nurseries in comparison with recommended dose of inorganic NPK were also standardized. VC with 20-25 encapsulated alginate beads (1g) was found optimum to enhance the growth and overall performance of black pepper cuttings and cardamom seedlings
- **The main aim of the entire work was to develop a stable, eco friendly and easy to carry formulation with consortia of indigenous biofertilizers and the attempt is a new area as far as black pepper and cardamom nutrition is concerned. The study led to the development of a consortium of efficient indigenous biofertilizers holding N fixing, P and K solubilizing bacteria encapsulated in sodium alginate beads as a formulation specifically for black pepper and cardamom nursery and standardized the organic substrate which can release the organisms easily when introduced into the rhizosphere.**
- Further research is required to study the performance of these efficient isolates in the form of consortia in combination with other inputs to improve growth of plants under various field conditions

## 8. REFERENCES


Ahmet E, Hiiseyin K, Sezai E, Metin T and Fikrettin S. 2003. The effect of spraying a growth promoting bacterium on the yield, growth and nutrient element composition of leaves of apricot. *Australian Journal of Agricultural Research*. 54(4):377-380.

Alagawadi A R and Gaur A C. 1992. Inoculation of Azospirillum *brasilense* and phosphate-solubilizing bacteria on yield of sorghum [*Sorghum bicolor* (L.) Moench] in dry land. *Tropical Agriculture.* 69: 347 - 350.

All India Coordinated Research Project on Spices (AICRPS). (2007). Annual report. *Indian Institute of Spices Research, Calicut, Kerala, India*.

Anandaraj M. 2000. Diseases of black pepper (In) Ravindran P N (Eds) Black pepper-*piper nigrum* L., medicinal and aromatic plants. *Harwood academic publishers. Netherlands* p 239-267.

Anith K N, Radhakrishnan N V and Manomohandas T P. 2002. Management of nursery wilt of black pepper (*piper nigrum* L.) with antagonistic bacteria. *Current Science* 83:561-562.

Arti D, Varsha N and Patel H H .2003. Halotolerent *Pseudomonas* sp. as TCP solubiliser. *Proceedings of the First National Symposium on MPS*. p 53-57.

Babana A H and Antoun H .2005. Biological system for improving the availability of Tilemsi phosphate rock for wheat cultivated in Mali. *Nutrient cycling in Agro ecosystems*. 72: 147-157.

Bahme J B, Schroth M N, VanGundy S D, Weinhold A R and Tolentino D M. 1988. Effect of inocula delivery systems on rhizobacterial colonization of underground organs of potato. *Phytopathology*.78:534-542.

Baldani J I, Krieg N R, Divan-Baldani V L, Hartmann A and Dobereiner J. 2005. (In) Bergey's Manual of Systematic Bacteriology. (Eds) 2nd .*Springer. New York. Berlin. Heidelberg*. p 7–26.

Barea JM, Azcon R and Azcon A C. 2004. Mycorrhizal fungi and plant growth promoting rhizobacteria (In) Varma A., Abbott L., Werner D and Hampp R (Eds) Plant surface microbiology. *Heidelberg. Germany: Springer-Verlag*.p 351–371.

Bashan Y and de Bashan L E. 2005. Bacteria (In) Hillel D (Eds) Encyclopedia of Soils in the Environment. *Elsevier. UK*. p 103–115.

Bashan Y, Holguin G and de Bashan LE. 2004. Azospirillum-plant relationships: physiological, molecular, agricultural, and environmental advances (1997-2003). *Canadian Journal of Microbiology*. 50(8):521-77.

Bashan Y, Juan P H, Luis A L and Macario B. 2002. Alginate micro beads as inoculants carriers for plant growth promoting bacteria. *Biology and Fertility of Soils*.35:359-368.

Bopaiah B M and Abdul Khader K B. 1989. Effect of biofertilizers on growth of black pepper (*Piper nigrum)*. *Indian Journal of Agricultural Sciences*. 59(10):682-683.

Bowen GD and Rovira AD. 1999. The rhizosphere and its management to improve plant growth. *Advances in Agronomy*. 66: 101–102.

Bremner J M. 1996. Nitrogen-total. In: Spark D.L. (Ed.) Methods of Soil analysis (Part 3) Chemical methods . *American society of agronom., Madison. USA*. p 1085-1121.

Chandra K and Singh T. 1999. (In) Post and present scenario of RBDC. Biofertilizer situation in Orissa. *NISTHAA. Bhubaneswar*. p 1-7.

Chiu C Y. 2005. Development and Application of Biofertilizers in Taiwan. http://www.agnet.org/library/eb/565/.

Day J M and Dobereiner J. 1976. Physiological aspects of $N_2$-fixation by a *spirillum* from *Digit aria* roots. *Soil Biology and Biochemistry*. 8: 45-50.

Deepa R, Alagawadi A R, Krishnaraj P U and Maheshkumar K S. 2003. Mineral phosphate solubilization by fluorescent pseudomonades*. Proceedings of the First National Symposium on MPS*. p 46-52.

Diby P, Sarma Y R, Srinivasan V and Anandaraj M. 2005. Pseudomonas fluorescens mediated vigour in black pepper (*Piper nigrum* L.) under green house cultivation*. Annals of Microbiology*. 55 (3):171-174.

Enrique A R C. 1982. Improved medium for the isolation of *Azospirillum* spp.*Applied and Environmental Microbiology*. 44:990-991.

Fallik E, Sarig S and Okon Y. 1994. Morphology and physiology of plant roots associated with Azospirillum (In) Okon Y (Eds) Azospirillum–Plant Associations. *CRC Press. Boca Raton. FL*. p 77–84.

Gaind S. 2004. Evaluation of fly ash as a carrier for diazotrophs and phospho bacteria. *Bioresource Technology*. 95(2): 187-190.

Govindan M and Chandy K C. 1985. Utilization of the diazotroph, *Azospirillum* for inducing rooting in pepper cuttings (*Piper nigrum*.L). *Current Science*. 54(22): 1186-1187.

Gyaneshwar P, Naresh Kumar G, Parekh L J and Poole P S. 2002. Role of soil microorganisms in improving P nutrition of plants*. Plant and Soil.* 245: 83-93.

Hamza S, Srinivasan V and Dinesh R. 2007. Nutrient diagnosis of black pepper gardens of Kerala and Karnataka. *Journal of spices and aromatic crops*. 16(2):77-81.

Han H S and Lee K D. 2006. Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant Soil and Environment* .52: 130-136.

Helsel Leta O, Hollis Dannie G, Steigerwalt Arnold G and Levett Paul N. 2006. Reclassification of *Roseomonas fauriae* Rihs *et al*. 1998 as a later heterotypic synonym of *Azospirillum brasilense* Tarrand *et al*. 1979. *International Journal of Systematic and Evolutionary Microbiology*. 56(12):2753-2755.

Hui L, Xiao Q W, Jia-Hong R and Jian-Ren Y E. 2011.Isolation and Identification of Phosphobacteria in Poplar Rhizosphere from Different Regions of China. *Pedosphere* . 21(1): 90-97.

Ivanova E, Teunou E and Poncelet D. 2002. Encapsulation of nitrogen fixing bacteria for biofertiliser production. *Department of food process engineering. France.*

Jackson M L. 1973. Soil Chemical Analysis*, Prentice Hall of India Pvt Ltd, New Delhi.*

Jagadeesh K S. 2003. MPS activity and biocontrol potential of fluorescent pseudomonads. *Proceedings of the First National Symposium on MPS* .p 41-45.

Kamble P U, Ramiah M and Patil, D V. 2011. Studies on compatibility of *Azospirillum*, *Pseudomonas fluorescens* and phosphobacteria for paddy seed inoculation. *Journal of Soils and Crops.* 10(2): 217-220.

Kandiannan K, Sivaraman K, Anandaraj M and Krishnamoorthy K S. 2000. Growth and nutrient content of black pepper cuttings as influenced by inoculation with biofertilisers. *Journal of Spices and Aromatic Crops*.9 (2):145-147.

Kanthaswamy V, Pillai O A, Natarajan S and Thamburaj S. 1996. Studies on nutrient requirement of black pepper (var.Panniyur1).*South Indian Horticulture*.44:3-4.

Kavitha R, Srinivasan V, Hamza S and Anandaraj M. 2007. Phosphate solubilizing bacteria isolated from rhizosphere soil and its growth promotion on black pepper. *First international Meeting on Microbial Phosphate Solubilization. Springer. Netherlands*.102:325-331.

Kennedy A C. 1998. The rhizosphere and spermosphere (In) Sylvia DM., Fuhrmann JJ., Hartel PG and Zuberer DA (Eds) Principles and applications of soil microbiology. *Upper Saddle River. New Jersey: Prentice Hall*.p 389–407.

Kennedy A C and Smith K L. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil* .170: 75–86.

Korikanthimath V S. 2000. Studies on chemical composition of coffee pulp and organic recycling in the coffee and cardamom cropping systems. *Crop Research* 20: 350-54.

Kumar A, Sarma Y R and Anandaraj M. 2004. Evaluation of genetic diversity of *Ralstonia solanaceraum* causing bacterial wilt of ginger using REP-PCR and PCR-RFLP. *current science*.87 (11):1555-1561.

Lucy M, Reed E and Glick B R. 2004. Application of free living plant growth-promoting rhizobacteria. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology.* 86: 1–25.

Marjan D B, Lentse Van D S, Leendert C and Peter A H M. 1999. Combining fluorescent *Pseudomonas* sp. strains to enhance suppression of *fusarium* wilt of radish. *European journal of Plant Pathology.*105(2):201-210.

Nautiyal C S. 1999. An efficient microbiological growth medium for screening phosphate-solubilizing microorganisms. *FEMS Microbiology Letters* .170: 265-270.

Nayak B. 2001. Uptake of potash by different plants with the use of potash mobilizing bacteria *Frateuria aurantia*. PhD thesis submitted to Orissa University of Agricultural science and Technology, *Bhubaneswar, India*.

Nguyen C, Yan W, Tacon F L and Lapeyrie F. 1992. Genetic variability of phosphate solubilizing activity by monocaryotic and dicaryotic mycelia of *Laccaria bicolor. Plant and Soil*.143:193-199.

Nybe E V, Nair P C S and Wahid P A. 1989. Relationships of foliar nutrient levels with yield in black pepper (*Piper nigrum* L.). *Tropical agriculture (Trinidad).* 66:345-349.

Nybe E V and Filite S. 2003. Organic manures and biofertilizers on nutrient availability and yield of black pepper. *Journal of Tropical Agriculture*.41:52-55.

Pankaj T and Anita P. 2008. Recovery of plant growth promoting rhizobacteria from sodium alginate beads after three years following storage at $4^0$C.*Journal of Indian Microbiology and Biotechnology*.35:205-209.

Patil P L.and Madalageri M B. 2003. Use of rock phosphate and phosphate solubilisers in green chilli. *Proceedings of the First National Symposium on MPS*.p 97-100.

Pikovskaya R E. 1948. Mobilization of phosphorus in soil in connection with vital activities of some microbial species**.** *Microbiologiae*. 17: 362-370.

Power B , Liu X, Germaine K J, Ryan D, Brazil D and Dowling D N. 2011. Alginate beads as a storage, delivery and containment system for genetically modified PCB degrader and PCB biosensor derivatives of *Pseudomonas fluorescens* F113. *Journal of Applied Microbiology* .Article first published online: 4 APR 2011.

Prasanna A V, Deepa P B, Murthy M, Deecaraman R Sridhar and Dhandapani P. 2011. Insoluble phosphate solubilization by bacterial strains isolated from rice rhizosphere soils from Southern India. *International Journal of Soil Science*. 6: 134-141.

Preininger E, Gyur J, Istuan B, Karoly and Pal. 2003. A new approach biolistic method, for the bombardment of living nitrogen fixing bacteria in to plant tissues. *In vitro Cellular and Developmental Biology*.39 (5): 443-449.

Ranganathan V and Natesan S. 1985. Cardamom soils and manuring. *Planters Chronicle*. 80(7):233-236.

Ramarethinam S and Krishan C. 2006. Studies on the effect of potash solubilizing/mobilizing bacteria *Frateuria aurantia* on brinjal (*Solanum melongena* L) growth and yield. *Pestology* .30:35-39.

Ravi G and Alagawadi A R. 2003. Effect of P-solubilizing biofertilizers on growth and nutrient uptake of sunflower. *Proceedings of the First National Symposium on MPS*. p 87-93.

Rekha P D, Wai A L, Arun A B and Chui C Y. 2007. Effect of free and encapsulated *Psedomonas putida* CC-FR2-4 and *Bacillus subtilis* CC-pg104 on plant growth under gnotobiotic conditions. *Bioresource Technology*.98:447-451.

Sadanandan A K .1994. Nutrition of black pepper, *Advance in horticulture, Kerala, India*.

Sarma Y R, Anandaraj M and Venugopal M N. 2000. Present and future strategies of disease management of black pepper (In) Diseases of plantation crops, spices, betalvine and mulberry (Eds). *Indian Institute of Spices Research. Calicut* . p 84-94.

Sarwar M and Kremer R J. 1995. Enhanced suppression of plant growth through production of L-TRP-derived compounds by deleterious rhizobacteria. *Plant and Soil* .172:261-269.

Sheng X F. 2005. Growth promotion and increased potassium uptake of cotton and rape by a potassium releasing strain of *Bacillus edaphicus*. *Soil Biology and Biochemistry.* 37:1918-1922.

Shrivastav S,Yadav K S and Kundu B S. 2004. Prospects of using phosphate solubilizing Pseudomonas as Biofungicide. *Indian Journal of Microbiology*.44:91-94.

Somasegaran P. 1985. Inoculant production with diluted liquid cultures of *Rhizobium* sp. and autoclaved peat: Evaluation of dilutents, *Rhizobium* sp., peats, sterility requirements, storage and plant effectiveness. *Applied and Enviornmental Microbiology*. 50 (2): 398-405

Somers E, Vanderleyden J and Srinivasan M. 2004. Rhizosphere Bacterial Signalling: A Love Parade Beneath Our Feet. *Critical Reviews in Microbiology*.30 (4):205-240.

Sompong M, Supamard P, Shotaro A and Tadashi Y. 2004. Phylogenetic and physiological characterization of indigenous *Azospirillum* isolates in Thailand. *Soil Science and Plant Nutrition*.50 (3):413-421.

Srinivasan K and Mary M V. 1981. Studies on fixation of phosphrous in acid soils of cardamom. *Journal of institution of chemists(India).* 53:145-156.

Srinivasan K. 1984. Organic carbon, total and available nitrogen in relation to cardamom soils. *Journal of institution of chemists (India).* 56:15-16.

Stackebrandt E and Goebel B M. 1994. A place for DNA-DNA reassociation and 16srRNA sequence analysis in the present species definition in bacteriology. *International Journal of systematic bacteriology* .44:846-849.

Trimurtulu N, Lalitha Kumari A and Latha M. 2011. Influence of mixed microbial consortium on plant growth and yields of chilli in vertisols. *International Journal of Current Research.* 2(1):61-66.

Thomas J and Kuruvila K M. 2007. Cardamom (In) *Proceedings of the national symposium on spices and aromatic crops* (SYMSAC IV). Dinesh R, Shiva K N, Prasath D, Nirmal Babu K and Parthasarthy V A (Eds). *OUAT. Bhubaneswar*. p 186-207.

Thomas J and Suseela Bhai R. 1990. Diseases of small cardamom and their management, Cardamom production technology, *ICRI, India*.

Thomas J, Suseela bhai R and Naidu R. 1991. Management of rot disease of cardamom through bioagents (Abs). National seminar on biological control of plantation crops. *RRII .Kottayam.Kerala*. p 21.

Thomas J, Suseela Bhai R, Dhanpal K and Vijayan A K. 1997. Integrated nutrient management of rot disease of cardamom .International conference on integrated plant disease management for sustainable agriculture*. New Delhi.*

Vance C P, Uhde-Stone C and Allan DL. 2003. Phosphorus acquisition and use: Critical adaptations by plants for securing a nonrenewable resource. *New Phytopathology* .157: 423–447.

Viveganandgan G and Jauhri K S. 2000. Growth and survival of phosphate solubilizing bacteria in calcium alginate. *Microbiology Research*.155:205-207.

Wose C R. 1987. Bacterial evolution .*Microbial Reverence*.51:221-271.

Wu S C, Cao Z H, Li Z G, Chung KC and Wong M H. 2005. Effects of biofertilizer containing N-fixer and K solubilizers and AM fungi on maize growth: a greenhouse trial. *Geoderma* .125:155-166.

**PUBLICATIONS**

**Sangeeth K P**, Suseela Bhai R, Srinivasan V and Venugopal M N. 2007. Comparative efficacy of indigenous microbial inoculants in improving seedling growth of cardamom. *National conference on organic waste utilization and ecofriendly technologies for Crop protection at NBPGR Regional station, Rajendranagar, Hyderabad.* p171-175.

**Sangeeth K P**, Suseela Bhai R and Srinivasan V. 2007. Isolation and selection of indigenous *Azospirillum* inoculants for their influence on growth and nutrient uptake rooted cuttings of Black pepper (*Piper nigrum* L). *National symposium on Spices and Aromatic crops (SYMSAC IV) at Orissa university of agriculture and technology (OUAT), Bhubaneswar.* p 333.

**Sangeeth K P**, Suseela Bhai R and Srinivasan V. 2008. Evaluation of Indigenous *Azospirillum* isolates for growth promotion in black pepper (*Piper nigrum* L.) rooted cuttings. *Journal of Spices and Aromatic crops* 17(2): 128-133.

**Sangeeth K.P** and Suseela Bhai R. 2009. *Invitro* plant growth-promoting and antagonistic potential of indigenous nitrogen fixers phosphate solubilizers and potassium mobilizers of black pepper (*piper nigrum* L). *5th International Conference on Plant Pathology in the Globalized Era. Indian Phytopathological Society. New Delhi*. p 348.

**Sangeeth K P**, Suseela Bhai R and Srinivasan V. 2010. Mobilization of Potassium from wood ash by *Paenibacillus glucanolyticus*: increased growth and K uptake of black pepper (*Piper nigrum* L.). *Journal of Spices and Aromatic crops*. (Communicated).

**Sangeeth K P** and Suseela Bhai R. 2010. Development and formulation of effective biofertilizers for management of black pepper and cardamom. *Symposium on changing plant disease scenario in relation to climate change. Indian Phytopathological Society (Southern Zone). IISR. Calicut*. p 37.

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